(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 December 2004 (16.12.2004)

PCT

(10) International Publication Number
WO 2004/108922 A2

(51) International Patent Classification[7]: C12N 15/00

(21) International Application Number: PCT/US2004/010965

(22) International Filing Date: 23 April 2004 (23.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/465,293 25 April 2003 (25.04.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND COMPOSITIONS FOR LOWERING TOTAL CHOLESTEROL LEVELS AND TREATMENT OF HEART DISEASE

(57) Abstract: Use of AAV7 and AAV8 to prepare a medicament for lowering total cholesterol levels and correcting defects in lipoprotein metabolism are described. These viral vectors carry an apolipoprotein A and/or E isoform. Use of these vectors in a combination regimen is also provided and kits useful therefor are described.

WO 2004/108922 A2

METHODS AND COMPOSTIONS FOR LOWERING TOTAL CHOLESTEROL LEVELS AND TREATMENT OF HEART DISEASE

STATEMENT OF FEDERALLY SPONSORED RESEARCH

Some of the work described in this application was performed under grants from the National Institutes of Health, Grant No. IP01-HL59407 and NIDDKGrant No. P30 DK 47757. The US government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Human apolipoprotein E (apoE) is a 34kDa polymorphic protein that plays a key role in lipoprotein metabolism and atherosclerosis. There are three isoforms of apoE. ApoE3 is the most common functional isoform. The adeno-associated virus (AAV) vectors, which have been isolated six serotypes, are promising vector for gene transfer in animals. Previous work implicates apoE3 transgene with traditional AAV, type 2 (AAV2-apoE3) vector by muscle injection [JD Harris *et al.*, *Gene Therapy*, **9**, 21-29 (2002)]. In their study, apoE3 transgene inhibited atherosclerosis, but no significant effect on plasma cholesterol in apoE$^{-/-}$ mice.

Human apolipoprotein A-I is another protein which has been implicated in lipoprotein metabolism. However, previous attempts to achieve meaningful systemic levels of apoA1 with an AAV vector have failed [SJ Chen, *et al, Mol Ther.* **2**:256-261 (2000)].

What is needed is a method of improving lipoprotein metabolism and lowering total cholesterol levels.

SUMMARY OF THE INVENTION

The present invention provides compositions useful in lowering total cholesterol levels, reducing total very low density lipoprotein (VLDL) levels, and/or raising high density lipoprotein (HDL) levels. The invention further provides compositions useful in treating atherosclerosis and heart disease, regulating elevated cholesterol levels and/or inappropriate lipoprotein metabolism.

Other aspects and advantages of the invention will be readily apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A through 1C are the amino acid sequences of the AAV7 [SEQ ID NO: 2] and AAV8 capsid vp1 proteins [SEQ ID NO:3], provided in alignment with the vp1 of the published sequences of AAV2 [SEQ ID NO:1], AAV1 [SEQ ID NO:4], and AAV3 [SEQ ID NO: 5] and newly identified AAV serotype AAV9 [SEQ ID NO: 6]. The alignment was performed using the Clustal W program, with the number of AAV2 used for reference. Underlining and bold at the bottom sequence of the alignment indicates cassettes of identity. The dots in the alignment indicate that the amino acids are missing at the positions in the alignment as compared to AAV2 VP1.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions for lowering total cholesterol levels, modulating lipoprotein metabolism, and treating atherosclerosis in a subject. These compositions contain an adeno-associated viral vector comprising a capsid protein selected from an AAV serotype (such as serotype 7 or serotype 8) which expresses sufficiently high levels of transgene that a therapeutically effective amount of an apolipoprotein upon delivery of AAV is achieved, preferably, a lower dose of AAV than is achieved by currently known AAV serotypes. The invention also provides kits useful for delivery of the compositions of the invention.

In one aspect, this method involves adeno-associated virus (AAV)-7 or AAV8-mediated delivery of a human apolipoprotein (apo) E or apoA under the control of regulatory control sequences which specifically directs expression of the gene in liver cells.

In another aspect, the invention provides compositions and regimens for reducing VLDL levels in a subject. This regimens involve AAV7 or AAV8-mediated delivery of a human apoE under the control of regulatory control sequences which specifically direct expression of the gene in liver cells. The invention also provides vectors, pharmaceutical compositions and kits useful for performing this method.

In still another aspect, the invention provides compositions and methods for raising total HDL levels in a subject. This method involves AAV7 or AAV8-mediated delivery of a human apoA under the control of regulatory control sequences which specifically directs expression of the gene in liver cells. The invention also provides vectors, pharmaceutical compositions and kits useful for performing this method.

In yet another aspect, the invention provides a regimen for treatment of a patient with elevated cholesterol levels and/or elevated VLDL levels. Such a regimen involves AAV7- and/or AAV8-mediated delivery of an apolipoprotein E. Optionally, such a regimen additionally involves AAV7- and/or AAV8-mediated delivery of an apoA. In addition, such a regimen may involve monitoring cholesterol levels and lipoprotein levels in the subject. This regimen, or variations thereof, can also be useful for prophylactic use in subjects at high risk for developing atherosclerosis or heart disease; and for treatment of subjects previously diagnosed with such conditions.

These and other embodiments and advantages of the invention are described in more detail below. As used throughout this specification and the claims, the terms "comprising" and "including" and their variants are inclusive of other components, elements, integers, steps and the like. Conversely, the term "consisting" and its variants are exclusive of other components, elements, integers, steps and the like.

I. Apolipoprotein E and Apolipoprotein A

Suitably, the vectors described herein are useful in the present invention to deliver an apoE and/or an apoA to a subject. Most suitably, for delivery to human subjects, human apolipoprotein is used. However, non-human mammalian apolipoproteins may be utilized as desired for treatment of human and non-human mammals.

Apo E and its isoforms 2, 3 and 4 have been described in the literature. These proteins, precursors, variants thereof, and the sequences encoding same are available from GenBank [see, e.g., accession number P02649], and from a variety of academic and commercial sources including, e.g., PanVera. In the examples herein, apoE

isoform 3 is utilized. However, another apoE isoform may be selected for use in the invention alone, or in combination with another apo isoform.

Similarly, apo A and its isoforms have been described in the literature. The apoA proteins, their precursors, and variants thereof, and the sequences encoding same, have been described in the literature, in GenBank [see, e.g., accession number LPHUA1], and from a variety of academic and commercial sources. In one desirable embodiment, ApoA isoform I (apo AI) is utilized in the invention. However, another apoA isoform may be selected for use in the invention alone, or in combination with another apo isoform as described herein.

Alternatively, an apoE or apoA may be isolated and purified using known techniques, e.g., those described in US Patent 6,423,803, US Patent 6,090,921; US Patent 5,672,685; and/or produced using known techniques. See, e.g., US Patent 5,116,739; Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY). Alternatively, an apolipoprotein, or a suitable fragment thereof, can be synthesized by well known solid phase peptide synthesis methods [e.g., Merrifield, *J. Am. Chem. Soc.*, **85**:2149 (1962); Stewart and Young, Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969) pp. 27-62)]. These and other suitable production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

II. Viral Vectors

The vectors useful in the present invention contain capsid proteins derived from AAV serotype 7 and/or AAV serotype 8. Such capsids include AAV7 and/or AAV8 capsid proteins, or fragments thereof, which retain the targeting and transfection ability of AAV7 and AAV8. Such capsids can be produced recombinantly, and include AAV7 and/or AAV8 capsids which have been modified for a variety of reasons, including, e.g.., to improve expression, yield or purification. Also included are viral vectors with capsid proteins which have been artificially generated by any suitable technique, using an AAV 7 or 8 sequence of the invention (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from another AAV serotype (known or novel), non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or

from a non-viral source. An artificial AAV7 or AAV8 capsid may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a "humanized" AAV capsid.

Thus, the vectors useful in the present invention can be produced using AAV7 and AAV8 nucleic acid sequences. The sequences of AAV7 and AAV8 have been described [G-P. Gao, *et al*, *Proc Natl Acad. Sci USA*, **99**(18):11854-11859 (Sep 3, 2002); GenBank database accession no. AF513851 (AAV7) and accession number AF513852 (AAV8). Additionally, AAV7 is the subject of a co-pending, co-owned US application, Serial No. 10/291,583, which is incorporated by reference herein, and AAV8 is the subject of co-owned PCT application No. PCT/US02/33630, filed November 12, 2002, which is also incorporated by reference herein as is its sequence listing. For serotype AAV7, the full-length nucleotide sequences, including the AAV 5' ITRs, capsid, rep, and AAV 3' ITRs; the amino acid sequences of the AAV7 capsid (Fig. 1), and the amino acid sequences of the AAV7 rep proteins [SEQ ID NO:9] are provided in the co-pending US Application No. 10/291,583. The AAV8 nucleic acid sequences of the invention include the DNA sequences, which consists of 4393 nucleotides; the amino acid sequences of the AAV8 capsid (Fig. 1); and the amino acid sequences of the AAV8 rep proteins [SEQ ID NO:10], which are provided in the co-owned PCT application PCT/US02/33630, which is incorporated by reference as is its Sequence Listing.

The AAV nucleic acid sequences useful in the invention further encompass the strand which is complementary to the coding strands, nucleic acid sequences, as well as the RNA and cDNA sequences corresponding to the sequences provided in the Sequence Listing, and their complementary strands. Also useful in the invention are natural variants and engineered modifications of the sequence listing of the above-references co-pending US and PCT applications, and their complementary strands. Such modifications include, for example, labels which are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with a degenerate nucleotide.

Further, nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98

to 99% identical or homologous to these sequences may be useful in the invention. These terms are as defined herein.

Also useful in the invention are fragments of the AAV7 and AAV8 sequences. Suitable fragments are at least 15 nucleotides in length, and encompass functional fragments, i.e., fragments that are of biological interest. In one embodiment, these fragments are fragments of AAV7 and AAV8 genomic DNA, their complementary strands, cDNA and RNA complementary thereto.

Using the alignment provided in the co-pending referenced US and PCT applications and in Fig. 1, (obtained using the Clustal W program at default settings), or similar techniques for generating an alignment with other novel serotypes of the invention, one of skill in the art can readily identify the precise nucleotide start and stop codons for desired fragments. As described herein, alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs, such as "Clustal W", accessible through Web Servers on the internet. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Similar programs are available for amino acid sequences, e.g., the "Clustal X" program. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program that provides at least the level of identity or alignment as that provided by the referenced algorithms and programs.

Examples of suitable fragments include the sequences encoding the three variable proteins (vp) of the AAV capsid which are alternative splice variants: vp1 [e.g., nt 825 to 3049 of AAV7 [SEQ ID NO:7]; nt 2121 to 4335 of AAV8, SEQ ID NO:8]; vp2 [e.g., nt 1234 - 3049 of AAV7, of SEQ ID NO:7; nt 2532 to 4335 of SEQ

ID NO:8]; and vp 3 [e.g., nt 1434 - 3049 of AAV7, SEQ ID NO:7; nt 2730 to 4335 of SEQ ID NO:8]. Other suitable fragments of AAV, include a fragment containing the start codon for the AAV capsid protein [e.g., nt 468 to 3090 of AAV7, SEQ ID NO:7, nt 725 to 3090 of AAV8, SEQ ID NO:8, and corresponding regions of the other AAV serotypes]. Still other fragments of AAV7 include those encoding the rep proteins, including *rep* 78, [e.g., initiation codon 334 for AAV7; initiation codon located at nt 227 of SEQ ID NO:8], *rep* 68 [initiation codon nt 334 for AAV7, SEQ ID NO:7; initiation codon located at nt 227 of SEQ ID NO:8], *rep* 52 [initiation codon 1006 of AAV7 of SEQ ID NO:7; initiation codon located at nt 905 of SEQ ID NO:8], and *rep* 40 [initiation codon 1006 of AAV7 of SEQ ID NO:7; initiation codon located at nt 905 of SEQ ID NO:8 for AAV8] Other fragments include the AAV 5' inverted terminal repeats (ITRs), [nt 1 to 107 of AAV7 for SEQ ID NO:7]; the AAV 3' ITRs [nt 4704 to 4721 of AAV7 of SEQ ID NO:7], P19 sequences, AAV P40 sequences, the rep binding site, and the terminal resolute site (TRS). Still other suitable fragments will be readily apparent to those of skill in the art. The corresponding regions in the other novel serotypes of the invention can be readily determined by utilizing conventional alignment techniques with the sequences provided herein.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over the full-length sequence, or an open reading frame thereof, or another suitable fragment which is at least 15 nucleotides in length. Examples of suitable fragments are described herein.

By the term "highly conserved" is meant at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. Identity is readily determined by one of skill in the art by resort to algorithms and computer programs known by those of skill in the art.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence,

or a fragment of at least about 500 to 5000 nucleotides. However, identity among smaller fragments, e.g., of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Similarly, "percent sequence identity" may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length, and may be up to about 700 amino acids. Examples of suitable fragments are described herein.

The invention provides proteins and fragments thereof which are encoded by the nucleic acid sequences of the novel AAV serotypes identified herein, including, e.g., AAV7 [nt 825 to 3049 of AAV7, SEQ ID NO:7].

The term "substantial homology" or "substantial similarity," when referring to amino acids or fragments thereof, indicates that, when optimally aligned with appropriate amino acid insertions or deletions with another amino acid, there is amino acid sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or a protein thereof, e.g., a cap protein, a rep protein, or a fragment thereof that is at least 8 amino acids, or more desirably, at least 15 amino acids in length. Examples of suitable fragments are described herein.

Particularly desirable proteins include the AAV capsid proteins, which are encoded by the nucleotide sequences identified above. The sequences of many of the AAV7 and AAV8 capsid proteins are provided in the Sequence Listing, SEQ ID NOs: 2 and 3, which is incorporated by reference herein. The AAV capsid is composed of three proteins, vp1, vp2 and vp3, which are alternative splice variants. The full-length sequence provided in these figures is that of vp1. Based on the numbering of the AAV7 capsid, the sequences of vp2 span amino acid 138 - 737 of AAV7 and the sequences of vp3 span amino acids 203 - 737 of AAV7.

The AAV8 capsid proteins include vpl [aa 1 to 737], vp2 [aa 138 to 737], and vp3 [aa 203 to 737] and functional fragments thereof. Examples of suitable fragments are amino acids 1 to 184, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736; aa 185 - 198; aa 260-273; aa447-477; aa495-602; aa660-669; and aa707-723, [SEQ ID NO:3]

Additionally, examples of other suitable fragments of AAV capsids include, with respect to the numbering of AAV2 [SEQ ID NO:1], aa 24 – 42, aa 25 – 28; aa 81 – 85; aa133-165; aa 134 – 165; aa 137-143; aa 154-156; aa 194-208; aa 261-274; aa 262-274; aa 171-173; aa 413-417; aa 449-478; aa 494-525; aa 534-571; aa 581-601; aa 660-671; aa 709-723. Still other desirable fragments include, for example, in AAV7, amino acids 1 to 184, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736; aa 185 to 198; aa 260 to 273; aa447 to 477; aa495 to 602; aa660 to 669; and aa707 to 723, of SEQ ID NO:2.

Other desirable proteins and fragments of the capsid protein include the constant and variable regions, located between hypervariable regions (HPV) and the sequences of the HPV regions themselves. An algorithm developed to determine areas of sequence divergence in AAV2 has yielded 12 hypervariable regions (HVR) of which 5 overlap or are part of the four previously described variable regions. [Chiorini *et al*, *J. Virol*, **73**:1309-19 (1999); Rutledge *et al*, *J. Virol.*, **72**:309-319] Using this algorithm and/or the alignment techniques described herein, the HVR of the novel AAV serotypes are determined. For example, utilizing Figure 1 and the alignments referenced herein, one can readily determine that for AAV7 [SEQ ID NO:2], HVR1 is located at aa 146 – 152; HVR2 is located at 182-187; HVR3 is located at aa 263-266, HVR4 is located at aa 383-385, HVR5 is located at aa 451-475; HVR6 is located at aa 491-496 of AAV7; HVR7 is located at aa 501-505; HVR8 is located at aa 513-521; HVR9 is located at 533-554; HVR10 is located at aa 583-596; HVR11 is located at aa 660-669; HVR12 is located at aa 707-721.

Still other desirable fragments include, for example, in AAV7 [SEQ ID NO:2], amino acids 1 to 184, amino acids 199 to 259; amino acids 274 to 446; amino acids 603 to 659; amino acids 670 to 706; amino acids 724 to 736; aa 185 to 198; aa 260 to 273; aa447 to 477; aa495 to 602; aa660 to 669; and aa707 to 723. Still other desirable regions, based on the numbering of AAV7, are selected from among the group consisting of aa 185 to 198; aa 260 to 273; aa447 to 477; aa495 to 602; aa660 to 669; and aa707 to 723. Using the alignment provided herein performed using the Clustal X program at default settings, or using other commercially or publicly

available alignment programs at default settings, one of skill in the art can readily determine corresponding fragments of the novel AAV capsids of the invention.

Other desirable proteins are the AAV rep proteins [aa 1 to 623] and functional fragments thereof, including, e.g., aa 1 to 171, aa 172 to 372, aa 373 to 444, aa 445 to 623 of SEQ ID NOs:9 and 10, among others. Still other desirable AAV7 and AAV8 rep proteins [SEQ ID NOs: 9 and 10] include rep68/78 and rep40/52 [located within aa 1 to 625 of SEQ ID NOs: 9 and 10]. Suitable fragments of the rep proteins may include aa 1 to 102; aa 103 to 140; aa 141 to 173; aa 174 to 226; aa 227 to 275; aa 276 to 374; aa 375 to 383; aa 384 to 446; aa 447 to 542; aa 543 to 555; aa 556 to 625.

Suitably, such fragments are at least 8 amino acids in length. In addition, fragments of other desired lengths can be readily utilized. Such fragments may be produced recombinantly or by other suitable means, *e.g.*, chemical synthesis.

The invention is not limited to use of AAV7 and AAV8 amino acid sequences, peptides and proteins expressed from the AAV7 and AAV8 nucleic acid sequences identified herein, but further encompasses the AAV7 and AAV8 amino acid sequences, peptides and proteins described herein, which are expressed from other nucleic acid sequences coding for these AAV7 and AAV8 amino acid sequences, peptides and protein (e.g., sequences which have been optimized for expression in the selected production cell), as well as amino acid sequences generated by other methods known in the art, including, e.g., by chemical synthesis, by other synthetic techniques, or by other methods.

In another embodiment, vectors may be generated from other AAV serotypes for delivery of an apolipoprotein. Such vectors may be used alone or in regimens utilizing the AAV.apo vectors described herein. Suitable AAV serotypes include such known serotypes as are described herein. Other suitable AAV serotypes include newly identified AAV serotypes such as are described in pending US Patent Application No. 10/291,583, incorporated by reference herein. Particularly desirable AAV serotypes include those with serotypes from serotypes which deliver transgenes in a manner which provides increased transgene expression in liver as compared to AAV serotypes 1-6. In one desirable embodiment, the selected AAV serotype allows expression of a sufficiently high amount of a transgene that a therapeutically effective

amount of transgene expression is obtained upon delivery of a low dose of AAV. Preferably, the AAV serotype selected demonstrates tropism for liver, as compared to other tissues or organs.

Additionally, AAV vectors carrying transgenes for co-expression with an apolipoprotein as described herein may be generated from AAV serotype 7, AAV serotype 8, known AAV serotypes 1-6, or the newly identified AAV serotypes provided in pending US Patent Application No. 10/291,583, incorporated by reference herein. Particularly desirable AAV serotypes include those serotypes which deliver transgenes in a manner which provides increased transgene expression as compared to AAV serotypes 1-6. However, the invention is not so limited. Further, such co-expressed transgenes may be expressed from another, non-AAV vector.

Suitable production techniques are well known to those of skill in the art. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY). Alternatively, peptides can also be synthesized by the well known solid phase peptide synthesis methods (Merrifield, *J. Am. Chem. Soc.*, **85**:2149 (1962); Stewart and Young, Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969) pp. 27-62). These and other suitable production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

III. Production of rAAV7 and rAAV8

The invention utilizes vectors with AAV capsids for delivery of an apolipoprotein A or apolipoprotein E. Vector containing the hexon protein of the AAV serotype as described herein is useful in the present invention. Such a vector can contain inverted terminal repeats (ITRs) from any selected AAV serotype, or may contain non-AAV sequences within its capsid, together with the apolipoprotein coding sequences which are preferably operably linked to a liver-specific promoter.

In one aspect, rAAV having a capsid derived from an AAV of serotype 7 capsid protein is generated for use in the invention. Such vectors can be produced using any suitable method. For example, a vector can be produced in a host cell which contains a nucleic acid sequence encoding an AAV serotype 7 capsid protein, or a fragment thereof, a functional rep gene; a minigene composed of, at a minimum,

AAV ITRs and an apo transgene; and sufficient helper functions to permit packaging of the minigene into the AAV7 capsid protein.

In another aspect, the rAAV having a capsid derived from an AAV of serotype 8 capsid protein is generated for use in the invention. Such vectors can be produced using any suitable method. For example, a vector can be produced in a host cell which contains a nucleic acid sequence encoding an AAV serotype 8 capsid protein, or a fragment thereof, a functional rep gene; a minigene composed of, at a minimum, AAV inverted terminal repeats (ITRs) and an apolipoprotein transgene; and sufficient helper functions to permit packaging of the minigene into the AAV8 capsid protein.

While the vectors useful in the present invention contain capsids of an AAV serotype described herein (e.g., AAV 7 and/or AAV8), the vectors may contain other elements from the same or other AAV serotypes, including any of the known serotypes 1, 2, 3, 4, 5 or 6 [see, American Type Culture Collection, Manassas Virginia and GenBank, among other sources] any of the novel serotypes described in co-pending US Patent application No. 10/291,583, or another selected AAV serotype. Such AAV elements, e.g., rep proteins, inverted terminal repeat sequences (ITRs), promoters/enhancers, and the like, may be obtained from a variety of commercial and academic sources including the ATCC, or produced synthetically from the sequences published in the literature, and computerized databases.

The components required to be cultured in the host cell to package an AAV minigene in an AAV capsid may be provided to the host cell in *trans*. Alternatively, any one or more of the required components (e.g., minigene, *rep* sequences, *cap* sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art. Most suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contains the rep and/or cap

proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

The minigene, *rep* sequences, *cap* sequences, and helper functions required for producing the rAAV used in the invention may be delivered to the packaging host cell in the form of any genetic element which transfers the transgene carried thereon. The selected genetic element may be delivered by any suitable method, including those described herein. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, *J. Virol.*, **70**:520-532 (1993) and US Patent 5,478,745.

A. The Minigene

The minigene is composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). It is this minigene which is packaged into a capsid protein and delivered to a selected host cell.

1. The transgene

The transgene is a nucleic acid sequence, heterologous to the vector sequences flanking the transgene, which encodes a polypeptide, protein, or other product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner that permits transgene transcription, translation, and/or expression in a host cell.

According to the present invention, the rAAV and/or rAAV vectors deliver the apolipoprotein transgene(s). Suitably, the apo transgene is selected from among an apolipoprotein E or apolipoprotein A, which is readily obtained from such sources as are described herein.

In still other embodiments, the invention may include delivery of lipases and/or receptor transgenes useful for modulation of cholesterol regulation and/or lipid modulation, optionally in a regimen that includes the apolipoprotein delivery method described herein. Examples of suitable lipases and/or

receptors include low density lipoprotein (LDL) receptor, the very low density lipoprotein (VLDL) receptor, high density lipoprotein (HDL) receptor, scavenger receptors (e.g., SRB1), inhibitors of cholesteryl ester transfer protein (CETP), and nuclear orphan receptor agonists that mediate expression of ATP-binding cassette transporter 1 (e.g., ABC1), and microsome triglyceride transfer protein, endothelial lipase, hepatic lipase, lipoprotein lipase, CD36, PLTP, LCAT, PPARα, PPARγ, PPARδ, LXRα, LXRβ, ApoA-IV, and ApoA-V.

Optionally, one or more of the transgenes described herein can be delivered by co-administering AAVs carrying the desired transgene(s), optionally which concatamerize *in vivo* to form a single vector genome. In such an embodiment, a first AAV may carry an expression cassette which expresses a single transgene and a second AAV may carry an expression cassette which expresses a different transgene for co-expression in the host cell. Alternatively, a first AAV may carry an expression cassette which is a first piece of a polycistronic construct (e.g., a promoter and ApoA1 (or ApoE3)) and a second AAV may carry an expression cassette which is a second piece of a polycistronic construct (e.g., LDLr/lipase and a polyA sequence). These two pieces of a polycistronic construct concatamerize *in vivo* to form a single vector genome which co-expresses the transgenes delivered by the first and second AAV. The invention further includes using multiple transgenes. In certain situations, a different transgene may be used to encode each subunit of a protein, or to encode different peptides or proteins. In order for the cell to produce the multi-subunit protein, a cell is infected with the recombinant virus containing each of the different subunits. Alternatively, different subunits of a protein (or isoforms of apoE and/or apoA) can be encoded by the same transgene. In this case, a single transgene includes the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribozyme entry site (IRES). This is desirable when the size of the DNA encoding each of the subunits is small, e.g., the total size of the DNA encoding the subunits and the IRES is less than five kilobases. As an alternative to an IRES, the DNA may be separated by sequences encoding a 2A peptide, which self-cleaves in a post-translational event. See, e.g., M.L. Donnelly, *et al, J. Gen. Virol.*, **78**(Pt 1):13-21 (Jan 1997); Furler, S., *et al, Gene Ther.*, **8**(11):864-873 (June 2001); Klump H., *et al., Gene Ther.*, **8**(10):811-817 (May 2001). This 2A peptide is significantly smaller than an IRES, making it well suited

for use when space is a limiting factor. However, the selected transgene may encode any biologically active product or other product, e.g., a product desirable for study.

Optionally, the vector may contain a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include, without limitation, DNA sequences encoding β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed thereto exist or can be produced by conventional means, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or Myc. These coding sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectrographic assays, fluorescent activating cell sorting assays and immunological assays, including enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and immunohistochemistry. For example, where the marker sequence is the LacZ gene, the presence of the vector carrying the signal is detected by assays for beta-galactosidase activity. Where the transgene is green fluorescent protein or luciferase, the vector carrying the signal may be measured visually by color or light production in a luminometer.

2. Regulatory Elements

In addition to the major elements identified above for the minigene, the vector also includes conventional control elements which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest.

Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA

processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

In a desired embodiment, the transgene is operably linked to a tissue-specific promoter. In the present invention, expression in liver is desired. Accordingly, liver-specific promoters are preferred. Examples of promoters that are tissue-specific for liver include (albumin, Miyatake *et al.*, *J. Virol.*, **71**:5124-32 (1997); hepatitis B virus core promoter, Sandig *et al.*, *Gene Ther.*, **3**:1002-9 (1996); alpha-fetoprotein (AFP), Arbuthnot *et al.*, *Hum. Gene Ther.*, 7:1503-14 (1996), and thyroxine-binding globulin (TBG) promoter [Wang, L., *et al.*, (1997) *Proc Natl Acad Sci U S A* **94**, 11563-11566], among others.

Optionally, the transgene (e.g., a marker or transgene coadministered with the apo transgene) may be operably linked to a constitutive promoter, an inducible promoter, a regulatable promoter, the native promoter for the selected transgene, or a tissue-specific promoter.

The combination of the transgene, promoter/enhancer, and 5' and 3' ITRs is referred to as a "minigene" for ease of reference herein. Provided with the teachings of this invention, the design of such a minigene can be made by resort to conventional techniques.

3. Delivery of the Minigene to a Packaging Host Cell

The minigene can be carried on any suitable vector, e.g., a plasmid, which is delivered to a host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and, optionally, integration in prokaryotic cells, mammalian cells, or both. These plasmids (or other vectors carrying the 5' AAV ITR-heterologous molecule-3' AAV ITR) contain sequences permitting replication of the minigene in eukaryotes and/or prokaryotes and selection markers for these systems. Selectable markers or reporter genes may include sequences encoding geneticin, hygromicin or purimycin resistance, among others. The plasmids may also contain certain selectable reporters or marker

genes that can be used to signal the presence of the vector in bacterial cells, such as ampicillin resistance. Other components of the plasmid may include an origin of replication and an amplicon, such as the amplicon system employing the Epstein Barr virus nuclear antigen. This amplicon system, or other similar amplicon components permit high copy episomal replication in the cells. Preferably, the molecule carrying the minigene is transfected into the cell, where it may exist transiently. Alternatively, the minigene (carrying the 5' AAV ITR-heterologous molecule-3' ITR) may be stably integrated into the genome of the host cell, either chromosomally or as an episome. In certain embodiments, the minigene may be present in multiple copies, optionally in head-to-head, head-to-tail, or tail-to-tail concatamers. Suitable transfection techniques are known and may readily be utilized to deliver the minigene to the host cell.

Generally, when delivering the vector comprising the minigene by transfection, the vector is delivered in an amount from about 5 μg to about 100 μg DNA, and preferably about 10 to about 50 μg DNA to about 1 x 10^4 cells to about 1 x 10^{13} cells, and preferably about 10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking into consideration such factors as the selected vector, the delivery method and the host cells selected.

B. *Rep* and *Cap* Sequences

In addition to the minigene, the host cell contains the sequences which drive expression of the AAV capsid protein (e.g., AAV7 or AAV8 capsid or an artificial capsid protein comprising a fragment of one or more of these capsids) in the host cell and rep sequences of the same serotype as the serotype of the AAV ITRs found in the minigene. The AAV *cap* and *rep* sequences may be independently obtained from an AAV source as described above and may be introduced into the host cell in any manner known to one in the art as described above. Additionally, when pseudotyping an AAV capsid for use in the invention, the sequences encoding each of the essential rep proteins may be supplied by the same AAV serotype, or the sequences encoding the rep proteins may be supplied by different AAV serotypes (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, or one of the serotypes identified herein). For example, the *rep*78/68 sequences may be from AAV2, whereas the *rep*52/40 sequences may from AAV1.

In one embodiment, the host cell stably contains the capsid protein under the control of a suitable promoter, such as those described above. Most desirably, in this embodiment, the capsid protein is expressed under the control of an inducible promoter. In another embodiment, the capsid protein is supplied to the host cell in *trans*. When delivered to the host cell in *trans*, the capsid protein may be delivered via a plasmid that contains the sequences necessary to direct expression of the selected capsid protein in the host cell. Most desirably, when delivered to the host cell in *trans*, the plasmid carrying the capsid protein also carries other sequences required for packaging the rAAV, e.g., the *rep* sequences.

In another embodiment, the host cell stably contains the *rep* sequences under the control of a suitable promoter, such as those described above. Most desirably, in this embodiment, the essential rep proteins are expressed under the control of an inducible promoter. In another embodiment, the rep proteins are supplied to the host cell in *trans*. When delivered to the host cell in *trans*, the rep proteins may be delivered via a plasmid that contains the sequences necessary to direct expression of the selected rep proteins in the host cell. Most desirably, when delivered to the host cell in *trans*, the plasmid carrying the capsid protein also carries other sequences required for packaging the rAAV, e.g., the *rep* and *cap* sequences.

Thus, in one embodiment, the *rep* and *cap* sequences may be transfected into the host cell on a single nucleic acid molecule and exist stably in the cell as an episome. In another embodiment, the *rep* and *cap* sequences are stably integrated into the genome of the cell. Another embodiment has the *rep* and *cap* sequences transiently expressed in the host cell. For example, a useful nucleic acid molecule for such transfection comprises, from 5' to 3', a promoter, an optional spacer interposed between the promoter and the start site of the *rep* gene sequence, an AAV *rep* gene sequence, and an AAV *cap* gene sequence.

Optionally, the *rep* and/or *cap* sequences may be supplied on a vector that contains other DNA sequences that are to be introduced into the host cells. For instance, the vector may contain the rAAV construct comprising the minigene. The vector may comprise one or more of the genes encoding the helper functions, e.g., the adenoviral proteins E1, E2a, and E4ORF6, and the gene for VAI RNA.

Preferably, the promoter used in this construct may be any of the constitutive, inducible or native promoters known to one of skill in the art or as discussed above. In one embodiment, an AAV P5 promoter sequence is employed. The selection of the AAV to provide any of these sequences does not limit the invention.

In another preferred embodiment, the promoter for *rep* is an inducible promoter, as are discussed above in connection with the transgene regulatory elements. One preferred promoter for *rep* expression is the T7 promoter. The vector comprising the *rep* gene regulated by the T7 promoter and the *cap* gene, is transfected or transformed into a cell which either constitutively or inducibly expresses the T7 polymerase. See WO 98/10088, published March 12, 1998.

The spacer is an optional element in the design of the vector. The spacer is a DNA sequence interposed between the promoter and the *rep* gene ATG start site. The spacer may have any desired design; that is, it may be a random sequence of nucleotides, or alternatively, it may encode a gene product, such as a marker gene. The spacer may contain genes that typically incorporate start/stop and polyA sites. The spacer may be a non-coding DNA sequence from a prokaryote or eukaryote, a repetitive non-coding sequence, a coding sequence without transcriptional controls or a coding sequence with transcriptional controls. Two exemplary sources of spacer sequences are the λ phage ladder sequences or yeast ladder sequences, which are available commercially, e.g., from Gibco or Invitrogen, among others. The spacer may be of any size sufficient to reduce expression of the *rep*78 and *rep*68 gene products, leaving the *rep*52, *rep*40 and *cap* gene products expressed at normal levels. The length of the spacer may therefore range from about 10 bp to about 10.0 kbp, preferably in the range of about 100 bp to about 8.0 kbp. To reduce the possibility of recombination, the spacer is preferably less than 2 kbp in length; however, the invention is not so limited.

Although the molecule(s) providing *rep* and *cap* may exist in the host cell transiently (i.e., through transfection), it is preferred that one or both of the *rep* and *cap* proteins and the promoter(s) controlling their expression be stably expressed in the host cell, e.g., as an episome or by integration into the chromosome of the host cell. The methods employed for constructing embodiments of this

invention are conventional genetic engineering or recombinant engineering techniques such as those described in the references above. While this specification provides illustrative examples of specific constructs, using the information provided herein, one of skill in the art may select and design other suitable constructs, using a choice of spacers, P5 promoters, and other elements, including at least one translational start and stop signal, and the optional addition of polyadenylation sites.

In another embodiment of this invention, the rep or cap protein may be provided stably by a host cell.

C. The Helper Functions

The packaging host cell also requires helper functions in order to package the rAAV of the invention. Optionally, these functions may be supplied by a herpesvirus. Most desirably, the necessary helper functions are each provided from a human or non-human primate adenovirus source, such as those described above and/or are available from a variety of sources, including the American Type Culture Collection (ATCC), Manassas, VA (US). In one currently preferred embodiment, the host cell is provided with and/or contains an E1a gene product, an E1b gene product, an E2a gene product, and/or an E4 ORF6 gene product. The host cell may contain other adenoviral genes such as VAI RNA, but these genes are not required. In a preferred embodiment, no other adenovirus genes or gene functions are present in the host cell.

The adenovirus E1a, E1b, E2a, and/or E4ORF6 gene products, as well as any other desired helper functions, can be provided using any means that allows their expression in a cell. Each of the sequences encoding these products may be on a separate vector, or one or more genes may be on the same vector. The vector may be any vector known in the art or disclosed above, including plasmids, cosmids and viruses. Introduction into the host cell of the vector may be achieved by any means known in the art or as disclosed above, including transfection, infection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion, among others. One or more of the adenoviral genes may be stably integrated into the genome of the host cell, stably expressed as episomes, or expressed transiently. The gene products may all be expressed transiently, on an episome or stably integrated, or some of the gene

products may be expressed stably while others are expressed transiently. Furthermore, the promoters for each of the adenoviral genes may be selected independently from a constitutive promoter, an inducible promoter or a native adenoviral promoter. The promoters may be regulated by a specific physiological state of the organism or cell (i.e., by the differentiation state or in replicating or quiescent cells) or by exogenously added factors, for example.

D. Host Cells And Packaging Cell Lines

The host cell itself may be selected from any biological organism, including prokaryotic (e.g., bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. Particularly desirable host cells are selected from among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, 293 cells (which express functional adenoviral E1), Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. The selection of the mammalian species providing the cells is not a limitation of this invention; nor is the type of mammalian cell, i.e., fibroblast, hepatocyte, tumor cell, etc. The most desirable cells do not carry any adenovirus gene other than E1, E2a and/or E4 ORF6; nor do they contain any other virus gene that could result in homologous recombination of a contaminating virus during the production of rAAV; and it is capable of infection or transfection of DNA and expression of the transfected DNA. In a preferred embodiment, the host cell is one that has *rep* and *cap* stably transfected in the cell.

One host cell useful in the present invention is a host cell stably transformed with the sequences encoding rep and cap, and which is transfected with the adenovirus E1, E2a, and E4ORF6 DNA and a construct carrying the minigene as described above. Stable *rep* and/or *cap* expressing cell lines, such as B-50 (PCT/US98/19463), or those described in U.S. Patent No. 5,658,785, may also be similarly employed. Another desirable host cell contains the minimum adenoviral DNA which is sufficient to express E4 ORF6. Yet other cell lines can be constructed using the novel AAV *rep* and/or novel AAV *cap* sequences of the invention.

The preparation of a host cell according to this invention involves techniques such as assembly of selected DNA sequences. This assembly may be accomplished utilizing conventional techniques. Such techniques include cDNA and genomic cloning, which are well known and are described in Sambrook et al., cited above, use of overlapping oligonucleotide sequences of the adenovirus and AAV genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods which provide the desired nucleotide sequence.

Introduction of the molecules (as plasmids or viruses) into the host cell may also be accomplished using techniques known to the skilled artisan and as discussed throughout the specification. In preferred embodiment, standard transfection techniques are used, e.g., $CaPO_4$ transfection or electroporation, and/or infection by hybrid adenovirus/AAV vectors into cell lines such as the human embryonic kidney cell line HEK 293 (a human kidney cell line containing functional adenovirus E1 genes which provides *trans*-acting E1 proteins).

The resulting rAAV7 or rAAV8 vectors are purified using techniques known to those of skill in the art, including multiple rounds of cesium chloride (CsCl) gradient centrifugation, or other suitable techniques such as those described [Gao *et al*, *Hu Gene Therapy*, **11**:2079-2091 (Oct 2002)] and elsewhere in the literature.

IV. Formulation of rAAV7.apo and rAAV8.apo

In another aspect, the present invention provides a method for delivery of an apolipoprotein transgene to a host that involves transfecting or infecting a selected host cell with a rAAV vector as described herein (e.g., rAAV7 or rAAV8). Methods for delivery are well known to those of skill in the art and are not a limitation of the present invention.

In one desirable embodiment, the invention provides a method for AAV7 and AAV8-mediated delivery of an apoE and/or an apoA transgene to a host. This method involves transfecting or infecting a selected host cell with a recombinant viral vector containing the selected transgene under the control of sequences that direct expression thereof.

Optionally, rAAV carrying different transgenes or a portion of a polycistronic cassette as described herein, is formulated for delivery to a host. Suitably, these different rAAV are formulated in a single composition. Alternatively, the rAAV may be delivered as separate compositions.

Any of the above-described recombinant vectors may be formulated for delivery to host cells according to published methods. The rAAV is mixed with a physiologically compatible carrier for administration to a human or non-human mammalian patient. Suitable carriers may be readily selected by one of skill in the art in view of the route(s) of delivery. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the present invention.

Optionally, the compositions of the invention may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the liver or lung, orally, intranasally, intratracheally, by inhalation, intravenously, intramuscularly, intraocularly, subcutaneously, intradermally, or by other routes of administration. Currently, intravenous and oral delivery routes are most desirable. However, other routes and combinations of different routes may be used, as desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral

vector is generally in the range of from about 1 ml to about 100 ml of solution containing concentrations of from about 1 x 10^7 to 1 x 10^{16} genomes virus vector. Suitably, because of the advantages conferred by the AAV7- and AAV8-mediated delivery according to the invention, preferred human doses are lower than those described with other vectors. For example, preferred human doses are in the range of about 10^{11} to 5 x 10^{11} to $10^{13}$to 5 x 10^{13} AAV genomes. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention.

V. Treatment Regimens and Pharmaceutical Kits

The invention provides treatment regimens for subjects having atherosclerosis, heart disease, or who are at high risk for developing these conditions. The invention further provides treatment regimens for patients having elevated cholesterol levels and/or lipoprotein metabolism disorders, including, e.g., elevated high density lipoprotein levels (HDL), undesirably low LDL (low density lipoprotein) levels, undesirable ratios of HDL to LDL, and/or elevated triglyceride levels.

In one aspect, the invention is useful for lowering total cholesterol levels in a subject by delivering to the subject a rAAV with a capsid derived from serotype 7 or 8 and which contains a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences, including a liver-specific promoter.

In one embodiment of this aspect, three isoforms of apoE, apoE2, apoE3, and apoE4, are delivered to the patient in a single delivery step. In other embodiments, only one apoE isoform is administered in a single delivery step. In yet another embodiment, rAAV7 or 8-mediated delivery of apoE is combined in a regimen involving repeat administration of the apoE isoform, a regimen involving multiple deliveries of an apoE or apoA, or a regimen involving delivery with other rAAV described herein, or with other medications. Suitably, the rAAV is delivered at a

lower dose than is typically known in the art for rAAV (i.e., the invention typically requires 10^{13} or fewer genome copies as compared to other AAV vectors which require $>10^{13}$-10^{16} genome copies). In one example, the apoE and the liver-specific promoter are on a cassette comprising AAV2 - 5' inverted terminal repeats (ITRs) and AAV2 - 3' ITRs in the AAV8 capsid. In another example, the apoE and the liver-specific promoter are on a cassette comprising AAV2 - 5' ITRs and AAV2 - 3' ITRs in the AAV7 capsid. However, the invention is not so limited.

In another aspect, the invention provides a method of reducing very low density lipoprotein (VLDL) levels in a subject by delivering to the subject a rAAV with a capsid derived from serotype 7 or 8 and which contains a gene encoding a human apolipoprotein E (apoE) under the control of a regulatory control sequences which direct expression of the gene, including a liver-specific promoter.

In still another aspect, the invention provides a method of raising high density lipoprotein (HDL) levels in a subject by delivering to the subject a rAAV with a capsid derived from serotype 7 or 8 and which contains a gene encoding a human apolipoprotein E (apoA) under the control of a regulatory control sequences which direct expression of the gene, including a liver-specific promoter.

In yet a further aspect, the invention provides a method of treating atherosclerosis in a subject by delivering to the subject a rAAV with a capsid derived from serotype 7 or 8 and which contains a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences, including a liver-specific promoter. Optionally, this method further involves monitoring total cholesterol levels in the subject. Additionally, or alternatively, this method may further involve monitoring lipoprotein levels in the subject.

In one embodiment of this aspect, three isoforms of apoE, apoE2, apoE3, and apoE4, are delivered to the patient in a single delivery step. In other embodiments, only one apoE isoform is administered in a single delivery step. In yet another embodiment, rAAV7 or 8-mediated delivery of apoE is combined in a regimen involving repeat administration of the apoE isoform, a regimen involving multiple deliveries of an apoE or apoA, or a regimen involving delivery with other medications. Suitably, the rAAV is delivered at a lower dose than is typically known

in the art for rAAV (i.e., the invention typically requires 10^{13} or fewer genome copies as compared to other AAV vectors which require $>10^{13}$-10^{16} genome copies). In one example, the apoE and the liver-specific promoter are on a cassette comprising AAV2 5' and 3' inverted terminal repeats in the AAV8 capsid. In another example, the apoE and the liver-specific promoter are on a cassette comprising AAV2 5' and 3' inverted terminal repeats in the AAV7 capsid.

In one aspect, the invention provides a treatment regimen involving rAAV and/or rAAV-mediated delivery of an apoE or apoA. As described herein, a subject may receive rAAV7-or rAAV8 in a regimen that also involves delivering rAAV comprising apoE. Suitably, the rAAV containing apoA is delivered at the same time as the rAAV comprising apoE, and wherein the rAAV comprise capsids of the same serotype. Where desired, the delivery of rAAV comprising apoA and rAAV comprising apoE is repeated using rAAV having capsids of a different serotype.

A treatment regimen as described herein can be used prophylactically. A regimen of the invention can further involve a combination therapy involving one or more rAAV7 and/or rAAV8 vectors delivering an apoE, an apoA, or combinations thereof, and administration of one or more of the vectors or transgenes described herein. Optionally, one or more of these delivery steps may be repeated. Additionally, such a combination therapy can optionally include a step of delivering an apoE and/or apoA transgene via another vector or other transport means. Such a vector can be a rAAV having a capsid of a serotype different from AAV7 or AAV8. Alternatively, the vector may be another viral or non-viral element that effectively delivers the apo to the target cells. In yet another alternative, a treatment regimen involves delivery of other active ingredients useful for treating subjects having atherosclerosis, heart disease, elevated cholesterol levels, or the other conditions described herein via rAAV or other delivery methods. Examples of additional active ingredients are known to those of skill in the art and may include, for example, statins, blood thinners, and the like.

In one aspect, the invention provides a kit useful in the treatment of a patient with elevated total cholesterol levels. Such a kit will include a container for the rAAV containing an apoE or apoA operably linked to a liver-specific promoter and formulated for delivery to the patient. Suitably, the rAAV is formulated for

intravenous delivery and further contains a needle, syringe, and instructions for administration. Alternatively, the rAAV is formulated for oral delivery as a gel cap, caplet or tablet, and contains blister packs or other appropriate packaging for the composition. Such a kit may further contain instructions for dosing. Optionally, the kit of the invention further contains instructions for an assay which monitors expression of the gene. Suitable assays for monitoring gene expression are known to those of skill in the art and are not a limitation of the present invention.

The following examples are illustrative of vectors and compositions of the invention, as well as those useful in the performance of the method of the invention. These examples are not intended to limit the present invention.

EXAMPLE 1 – LIVER TARGETED AAV7-ApoE3 and AAV8-E3 LOWER TOTAL CHOLESTEROL

Adeno-associated vectors with serotype 7 and 8 capsids were constructed which contained a cassette containing AAV2 5' inverted terminal repeats (ITRs), a liver-specific thyroid hormone-binding globulin (TBG) promoter [Wang, L., *et al.*, (1999) *Proc Natl Acad Sci U S A* **96**, 3906-3910, sequences encoding human apoE3, and AAV2 3' ITRs. (AAV2/7-apoE3 and AAV2/8-apoE3). AAV7 vectors were known to have good expression in skeletal muscle, thus the following findings regarding the ability of these vectors to express apoE in liver cells was unexpected. Although AAV8 vectors were known to have good expression in liver, their ability to express apoE3 in a manner which demonstrates a significant lowering in cholesterol levels was unexpected in view of a previous study in which apoE3 failed to lower cholesterol.

A. Construction of AAV cis-plasmid pAAV-TBG-ApoE3

A cis-plasmid that contains the TBG-ApoE3 cassette flanked by AAV2 inverted terminal repeats (ITRs) was constructed as follows A cis-plasmid that contains the TBG-ApoE3 cassette flanked by AAV2 inverted terminal repeats (ITRs) was constructed as follows. Human apoE3 cDNA was digested with KpnI and XhoI and inserted into the KpnI and SalI sites of the pAAVnewTBG-BI-rhepo-l vector; the resulting cis-plasmid is termed pAAV.TBG.apoE3.

B. Production of AAV2/7 and AAV2/8 TBG.ApoE3

AAV2/7 and AAV2/8 TBG.apoE3 were produced by triple transfection in combination with a pseudotyping strategy, followed by purification by three rounds of cesium chloride gradient centrifugation.

The three plasmids used are: AAV cis-plasmid pAAV TBG.apoE3 as described above, AAV trans plasmid that contains rep protein from AAV2 and capsid protein from AAV7 or AAV8 as described by Gao, G., et al., (2002) *Proc Natl Acad Sci USA* **99**, 11854-11859, and an adenoviral helper plasmid, termed pAdΔF6, which provides the essential helper functions of E2a and E4 ORF6 not provided by the E1-expressing 293 cell, but is deleted of adenoviral capsid proteins and functional E1 regions.

For each vector preparation, fifty 15-cm plates of subconfluent 293 cells (ATCC, grow in DMEM medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO_2) were transfected by calcium phosphate methodology. Cells were changed with fresh medium two hours before transfection.

The chimeric trans-plasmid for production of recombinant pseudotyped AAV7 vectors was produced as described [G. Gao, et al., (2002) *Proc Natl Acad Sci USA* **99**, 11854-11859]. More particularly, p5E18 plasmid (Xiao *et al.*, 1999, *J. Virol* **73**:3994-4003) was partially digested with Xho I to linearize the plasmid at the Xho I site at the position of 3169 bp only. The Xho I cut ends were then filled in and ligated back. This modified p5E18 plasmid was restricted with Xba I and Xho I in a complete digestion to remove the AAV2 cap gene sequence and replaced with a 2267 bp Spe I/Xho I fragment containing the AAV7 cap gene which was isolated from pCRAAV7 6-5+15-4 plasmid. The resulting plasmid contains the AAV2 rep sequences for Rep78/68 under the control of the AAV2 P5 promoter, and the AAV2 rep sequences for Rep52/40 under the control of the AAV2 P19 promoter. The AAV7 capsid sequences are under the control of the AAV2 P40 promoter, which is located within the Rep sequences. This plasmid further contains a spacer 5' of the rep ORF.

An adenoviral helper plasmid, termed pAdΔF6, provides the essential helper functions of E2a and E4 ORF6 not provided by the E1-expressing helper cell,

but is deleted of adenoviral capsid proteins and functional E1 regions). [See, G. Gao, et al., (2002) *Proc Natl Acad Sci USA* **99**, 11854-11859].

A transfection cocktail was made by first mixing 650 μg of cis-plasmid pAAV.TBG.apoE3, 650 μg of trans plasmid that contains AAV2 rep gene and AAV7 or AAV8 cap gene (p5E18-VD2/7 or p5E18-VD2/8), 1300 μg of adeno helper plasmid (pAdΔF6), 6.5 mL of 2.5 $CaCl_2$, and 59 mL of Milli-Q H_2O to generate a DNA-calcium mixture. Then 12.5 mL of DNA-calcium mixture was dripped to a 50 mL conical tube containing 12.5 mL of 2xHBS buffer (50 mM HEPES, 0.28 M NaCl, 1.4 mM Na_2HPO_4, pH 7.05) while vortexing. This was repeated for another 4 tubes. The completed cocktails were incubated at room temperature for 5 minutes for DNA-calcium phosphate precipitate to develop. Then 2.5 mL of the transfection cocktail was added to each plate. Medium was changed the following day, and cells were harvested 3 days after transfection.

For purification, cell pellet was resuspended in 27 mL of resuspension buffer (50 mM Tris, pH 7.4, 1 mM $MgCl_2$). Cells were disrupted by sonication. Cellular DNA was treated with 375000 units of Benzonase® nuclease [Merck, Germany] at 37 °C for 20 minutes. Clear cell lysate was generated after the deoxycholic acid treatment 0.5% DOC, 37 °C for 10 minutes) and centrifugation at 3000 rpm at 4 °C for 15 minutes. Then the clear lysate was loaded onto a 2-tier gradient consisting of 9 mL of 1.61 g/mL and 9 mL of 1.41 g/mL CsCl in Beckman® SW-29 [Beckman Coulter, CA] tubes, and centrifuged at 25,000 rpm at 15 °C for 18-20 hours. After the centrifugation, 1 mL-fractions containing vectors were collected. Fractions with refraction index reading between 1.3650 and 1.3760 were combined and loaded into two 70 Ti quick seal tubes and subjected to a second round of CsCl gradient centrifugation at 60,000 rpm at 15 °C for 20-24 hours. After the third round of CsCl centrifugation, fractions with refraction index reading between 1.3670 and 1.3740 were combined, desalted using the UltraFree® 100K centrifugal filter device [Millipore, MA, US] and washed twice with 10 mL of phosphate buffered saline (PBS). Genome copy titer of the vector prep was determined by TaqMan® probe (Applied Biosystems, CA, US) analysis by using probes and primers targeting SV40 polyA.

C. In Vivo Study - Mouse Model

In order to study the effect of apoE3 delivered as described herein on lipoprotein metabolism, a well-known mouse model was utilized. See, K. Tsukamoto et al, "Rapid regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein E in apoE deficient mice", *Arterioscler. Thromb. Vasc. Biol.*, 19:2162-2170 (1999). More particularly, the AAV7-apoE3 and AAV8-apoE3 vectors were injected via tail vein of $apoE^{-/-}$ mice. At day 14 after vector injection, mean total cholesterol levels in mice treated with AAV7-apoE and AAV8-apoE3 were 69mg/dl and 72mg/dl, respectively, and with the control vector was 839mg/dl. Lipoprotein profiles of plasma revealed substantial reductions in VLDL and remnant lipoproteins in mice treated with AAV7-apoE3 and AAV8-apoE3. Western blot for human apoE protein has shown expression of human apoE in mouse plasma up to six months. Human apoE levels peaked at 2-fold of human plasma levels on day 28. At day 180, total cholesterol levels in mice treated with AAV7-apoE3 and AAV8-apoE3 were still 12-fold lower than with control vector. Atherosclerosis in AAV7- and AAV8-apoE3 treated mice was completely prevented as evidenced at one year after injection. These results indicate that intravenous administration of AAV7 and AAV8 vectors mediated the expression and secretion of human apoE3 *in vivo*.

EXAMPLE 2 – AAV7 AND AAV8-MEDIATED HEPATIC EXPRESSION OF HUMAN APOA-I IN APOA-I DEFICIENT MICE

AAV8 based vectors have achieved levels of apoA1 which have been stable for at least six months and which are 20 fold-higher than those obtained with previously tested AAV vectors.

AAV2/7 and AAV2/8 vectors were constructed essentially as described above, with the exception that the vectors expressed human apolipoprotein A-I under the control of the TBG liver-specific promoter (as opposed to apoE3).

In studies in wild-type mice, both AAV2/7 and AAV2/8 vectors generated plasma levels of human apoA-I that were 100-150 mg/dL, similar to normal human plasma levels of apoA-I. Plasma human apoA-I levels are quantitated using an immunoturbidometric assay on the Cobas Fara II® analyzer (Roche Diagnostic Systems Inc., NJ, USA). To date, expression has extended beyond 3 months.

In a study using apoA-I deficient mice, the AAV2/7.TBG.apoAI and AAV2/8.TBG.apoAI vectors were injected intravenously via the tail vein. [Li H, et al., *Arterioscler Thromb*. 1993;**13**:1814-21]. On day 42 after vector injection, the mean apoA-I level in mice injected with the AAV7-apoA-I vector was 41.3 mg/dl, in those injected with AAV8-apoA-I was 52 mg/dl and in those injected with AAV2-apoA-I was 1.3 mg/dl. Correspondingly, these mice had mean HDL-C of 20, 23 and 8.7 mg/dl respectively. These results indicate that intravenous injection of AAV7-apoA-I and AAV8-apoA-I resulted in high-level expression and secretion of human apoA-I.

All publications cited in this specification are incorporated herein by reference. While the invention has been described with reference to particularly preferred embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims.

What is claimed is:

1. A method of lowering total cholesterol levels in a subject, said method comprising the step of delivering to the subject a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from an AAV serotype which preferentially expresses high levels of transgene in liver, said rAAV comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter,

wherein the rAAV capsid protein is selected from an AAV serotype which expresses sufficiently high levels of transgene in liver that a therapeutically effective amount of apoE or apoA expression is obtained upon delivery of a low dose of AAV.

2. A method of lowering total cholesterol levels in a subject, said method comprising the step of delivering to the subject a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from serotype 7 or 8, said rAAV comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter.

3. The method according to claim 1 or claim 2, wherein the apoE is isoform 3.

4. The method according to claim 1 or claim 2, comprising delivering to the subject isoforms 2, 3 and 4 of apoE.

5. The method according to claim 4, wherein apoE2, apoE3, and apoE4 are delivered to the patient in a single delivery step.

6. The method according to any of claims 1 to 5 further comprising the step of delivering to the subject a lipase or receptor transgene useful for modulation of cholesterol regulation and/or lipid modulation.

7. The method according to claim 6, wherein said lipase or receptor transgene is selected from the group consisting of: low density lipoprotein receptor, the very low density lipoprotein receptor, scavenger receptors, inhibitors of cholesteryl ester transfer protein, nuclear orphan receptor agonists that mediate expression of ATP-binding cassette transporter 1, microsome triglyceride transfer protein, endothelial lipase, hepatic lipase, lipoprotein lipase, CD36, PLTP, LCAT, PPARα, PPARγ, PPARδ, LXRα, LXRβ, ApoA-IV, and ApoA-V.

8. The method according to any of claims 2 to 7, wherein the rAAV is delivered at a low dose.

9. The method according to any of claims 1 to 8, wherein the apoA is isoform I (apoA-I).

10. The method according to any of claims 1 to 8, wherein the apoE and the liver-specific promoter are on a cassette comprising AAV2 5' and 3' inverted terminal repeats in the AAV8 capsid.

11. The method according to any of claims 1 to 8, wherein the apoE and the liver-specific promoter are on a cassette comprising AAV2 5' and 3' inverted terminal repeats in the AAV7 capsid.

12. The method according to any of claims 1 to 11, wherein the rAAV is delivered intravenously.

13. The method according to any of claims 1 to 11, wherein the rAAV is delivered orally.

14. The method according to any of claims 1 to 11, wherein the liver-specific promoter is thyroxine-binding globulin promoter.

15. A method of reducing very low density lipoprotein (VLDL) levels in a subject, said method comprising the step of delivering to the subject a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from serotype 7 or 8, said rAAV comprising a gene encoding a human apolipoprotein E (apoE) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter.

16. A method of raising high density lipoprotein (HDL) levels in a subject, said method comprising the step of delivering to the subject a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from serotype 7 or 8, said rAAV comprising a gene encoding a human apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter.

17. A method of treating atherosclerosis in a subject, said method comprising the step of delivering to the subject a recombinant adeno-associated virus (rAAV) comprising an capsid protein selected from serotype 7 or 8, said rAAV comprising a gene encoding a human apolipoprotein (apoE) or human apolipoprotein A under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter.

18. The method according to claim 17, further comprising the step of monitoring total cholesterol levels in the subject.

19. The method according to claim 17, further comprising the step of monitoring lipoprotein levels in the subject.

20. The method according to claim 17, said method comprising delivering to the subject rAAV comprising apoA in a regimen which also comprises delivering rAAV comprising apoE.

21. The method according to claim 20, wherein the rAAV comprising apoA is delivered at the same time as the rAAV comprising apoE, and wherein the rAAV comprise capsids of the same serotype.

22. The method according to claim 21, wherein the rAAV capsids are of serotype 7.

23. The method according to claim 21, wherein the step of delivering rAAV comprising apoA and rAAV comprising apoE is repeated using rAAV having capsids of a different serotype.

24. The method according to claim 23, wherein the rAAV capsids are of serotype 8.

25. Use of a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from an AAV serotype which preferentially expresses high levels of transgene in liver in preparing a medicament for lowering total cholesterol levels in a subject,

said rAAV comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter,

wherein the rAAV capsid protein is selected from an AAV serotype which expresses sufficiently high levels of transgene in liver that a therapeutically effective amount of apoE or apoA expression is obtained upon delivery of a low dose of AAV.

26. Use of a recombinant adeno-associated virus (rAAV) comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter, in a medicament for lowering total cholesterol levels in a subject; wherein said recombinant adeno-associated virus (rAAV) comprises a capsid protein selected from serotype 7 or 8.

27. Use of a combination of recombinant adeno-associated virus (rAAV) in preparing a medicament for lowering total cholesterol levels in a subjection, said combination comprising one or more rAAV encoding a human apolipoprotein E (apoE) under the control of a regulatory control sequences which direct expression of the gene and/or one or more rAAV encoding a human apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene.

28. Use according to any of claims 25 to 27, wherein the apoE is selected from isoform 3.

29. Use according to any of claims 25 to 27, wherein the apoE is selected from isoforms 2, 3 and 4 of apoE.

30. Use according to claim 27, wherein the combination comprises rAAV encoding apoE2, rAAV encoding apoE3, and rAAV encoding apoE4.

31. Use according to any of claims 25 to 30, wherein the medicament is co-administered in a regimen comprising a lipase or receptor transgene useful for modulation of cholesterol regulation and/or lipid modulation.

32. Use according to any of claims 27 to 30, wherein the medicament comprises a low dose of rAAV.

33. Use according to any of claims 25 to 27, wherein the apoA is isoform I (apoA-I).

34. Use according to any of claims 25 to 27, wherein the medicament is formulated for intravenous delivery.

35. Use according to any of claims 25 to 27, wherein the medicament is formulated for oral delivery.

36. A kit useful in the treatment of a patient with elevated total cholesterol levels comprising:

a container comprising a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from an AAV serotype which preferentially expresses high levels of transgene in liver, said rAAV comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter,

wherein the rAAV capsid protein is selected from an AAV serotype which expresses sufficiently high levels of transgene in liver that a therapeutically effective amount of apoE or apoA expression is obtained upon delivery of a low dose of AAV, said rAAV formulated for delivery to the patient.

37. A kit useful in the treatment of a patient with elevated total cholesterol levels comprising:

a container comprising a recombinant adeno-associated virus (rAAV) comprising a capsid protein selected from serotype 7 or 8, said rAAV comprising a gene encoding a human apolipoprotein E (apoE) or apolipoprotein A (apoA) under the control of a regulatory control sequences which direct expression of the gene, said regulatory control sequences comprising a liver-specific promoter, said rAAV formulated for delivery to the patient.

38. The kit according to claim 36 or claim 37, wherein the rAAV is formulated for intravenous delivery.

39. The kit according to claim 36 or claim 37, wherein the kit further comprises a needle, syringe, and instructions.

40. The kit according to claim 36 or claim 37, wherein the kit further comprises instructions for an assay which monitors expression of the gene.

Fig. 1A

```
          1                                                  50
AAV_2   MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY
AAV_7   MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD NGRGLVLPGY
AAV_8   MAADGYLPDW LEDNLSEGIR EWWALKPGAP KPKANQQKQD DGRGLVLPGY
AAV_1   MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY
AAV_3   MAADGYLPDW LEDNLSEGIR EWWALKPGVP QPKANQQHQD NRRGLVLPGY
AAV_9   MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY

         51                                                100
AAV_2   KYLGPFNGLD KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADAEF
AAV_7   KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF
AAV_8   KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLQAGDNPY LRYNHADAEF
AAV_1   KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF
AAV_3   KYLGPGNGLD KGEPVNEADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF
AAV_9   KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF

          101                                               150
AAV_2   QERLKEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEPVKTAP GKKRPVEHSP
AAV_7   QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP AKKRPVEPSP
AAV_8   QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEPSP
AAV_1   QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP
AAV_3   QERLQEDTSF GGNLGRAVFQ AKKRILEPLG LVEEAAKTAP GKKGAVDQSP
AAV_9   QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP

          151                                               200
AAV_2   .VEPDSSSGT GKAGQQPARK RLNFGQTGDA DSVPDPQPLG QPPAAPSGLG
AAV_7   QRSPDSSTGI GKKGQQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPSSVG
AAV_8   QRSPDSSTGI GKKGQQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPSGVG
AAV_1   .QEPDSSSGI GKTGQQPAKK RLNFGQTGDS ESVPDPQPLG EPPATPAAVG
AAV_3   .QEPDSSSGV GKSGKQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPTSLG
AAV_9   QE.PDSSSGI GKSGQQPAKK RLNFGQTGDS ESVPDPQPLG EPPEAPSGLG

          201                                               250
AAV_2   TNTMATGSGA PMADNNEGAD GVGNSSGNWH CDSTWMGDRV ITTSTRTWAL
AAV_7   SGTVAAGGGA PMADNNEGAD GVGNASGNWH CDSTWLGDRV ITTSTRTWAL
AAV_8   PNTMAAGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGDRV ITTSTRTWAL
AAV_1   PTTMASGGGA PMADNNEGAD GVGNASGNWH CDSTWLGDRV ITTSTRTWAL
AAV_3   SNTMASGGGA PMADNNEGAD GVGNSSGNWH CDSQWLGDRV ITTSTRTWAL
AAV_9   PNTMASGGGA PMADNNEGAD GVGNSSGNWH CDSTWLGDRV ITTSTRTWAL

          251                                               300
AAV_2   PTYNNHLYKQ ISSQS--GAS NDNHYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_7   PTYNNHLYKQ ISSETA-GST NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ
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AAV_3   PTYNNHLYKQ ISSQS..GAS NDNHYFGYST PWGYFDFNRF HCHFSPRDWQ
AAV_9   PTYNNHLYKQ ISNGTSGGST NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ
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Fig. 1B

```
        301                                                        350
AAV_2   RLINNNWGFR PKRLNFKLFN IQVKEVTQND GTTTIANNLT STVQVFTDSE
AAV_7   RLINNNWGFR PKKLRFKLFN IQVKEVTTND GVTTIANNLT STIQVFSDSE
AAV_8   RLINNNWGFR PKRLSFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE
AAV_1   RLINNNWGFR PKRLNFKLFN IQVKEVTTND GVTTIANNLT STVQVFSDSE
AAV_3   RLINNNWGFR PKKLSFKLFN IQVRGVTQND GTTTIANNLT STVQVFTDSE
AAV_9   RLINNNWGFR PKRLNFKLFN IQVKEVTTNE GTKTIANNLT STVQVFTDSE

        351                                                        400
AAV_2   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_7   YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQSV GRSSFYCLEY
AAV_8   YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_1   YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_3   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAV GRSSFYCLEY
AAV_9   YQLPYVLGSA HQGCLPPFPA DVFMVPQYGY LTLNNGSQAL GRSSFYCLEY

        401                                                        450
AAV_2   FPSQMLRTGN NFTFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR
AAV_7   FPSQMLRTGN NFEFSYSFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLAR
AAV_8   FPSQMLRTGN NFQFTYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR
AAV_1   FPSQMLRTGN NFTFSYTFEE VPFHSSYAHS QSLDRLMNPL IDQYLYYLNR
AAV_3   FPSQMLRTGN NFQFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLNR
AAV_9   FPSQMLRTGN NFQFSYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLVR

        451                                                        500
AAV_2   TNTPSG.TTT QSRLQFSQAG ASDIRDQS RNWLPGPCYRQQ RVSKTSADNN
AAV_7   TQSNPGGTAG NRELQFYQGG PSTMAEQA KNWLPGPCFRQQ RVSKTLDQNN
AAV_8   TQTTGG.TAN TQTLGFSQGG PNTMANQA KNWLPGPCYRQQ RVSTTTGQNN
AAV_1   TQ.NQSGSAQ NKDLLFSRGS PAGMSVQP KNWLPGPCYRQQ RVSKTKTDNN
AAV_3   TQGTTSGTTN QSRLLFSQAG PQSMSLQA RNWLPGPCYRQQ RLSKTANDNN
AAV_9   TQTTG..TGG TQTLAFSQAG PSSMANQA RNWVPGPCYRQQ RVSTTTNQNN

        501                                                        550
AAV_2   NSEYSWTGAT KYHLNGRDSL VNPGPAMASH KDDEEKFFPQ SGVLIFGKQG
AAV_7   NSNFAWTGAT KYHLNGRNSL VNPGVAMATH KDDEDRFFPS SGVLIFGKTG
AAV_8   NSNFAWTAGT KYHLNGRNSL ANPGIAMATH KDDEERFFPS NGILIFGKQN
AAV_1   NSNFTWTGAS KYNLNGRESI INPGTAMASH KDDEDKFFPM SGVMIFGKES
AAV_3   NSNFPWTAAS KYHLNGRDSL VNPGPAMASH KDDEEKFFPM HGNLIFGKEG
AAV_9   NSNFAWTGAA KFKLNGRDSL MNPGVAMASH KDDEDRFFPS SGVLIFGKQG

        551                                                        600
AAV_2   SEKTNVDIEK VMITDEEEIR T TNPVATEQY GSVSTNLQRG NRQAATADVN
AAV_7   ATNKTT-LEN VLMTNEEEIR P TNPVATEEY GIVSSNLQAA NTAAQTQVVN
AAV_8   AARDNADYSD VMLTSEEEIK T TNPVATEEY GIVADNLQQQ NTAPQIGTVN
AAV_1   AGASNTALDN VMITDEEEIK A TNPVATERF GTVAVNFQSS STDPATGDVH
AAV_3   TTASNAELDN VMITDEEEIR T TNPVATEQY GTVANNLQSS NTAPTTGTVN
AAV_9   AGNDGVDYSQ VLITDEEEIK A TNPVATEEY GAVAINNQAA NTQAQTGLVH
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Fig. 1C

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      601                                                        650
AAV_2 TQGVLPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKHPPPQI
AAV_7 NQGALPGMVW QNRDVYLQGP IWAKIPHTDG NFHPSPLMGG FGLKHPPPQI
AAV_8 SQGALPGMVW QNRDVYLQGP IWAKIPHTDG NFHPSPLMGG FGLKHPPPQI
AAV_1 AMGALPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKNPPPQI
AAV_3 HQGALPGMVW QDRDVYLQGP IWAKIPHTDG HFHPSPLMGG FGLKHPPPQI
AAV_9 NQGVIPGMVW QNRDVYLQGP IWAKIPHTDG NFHPSPLMGG FGLKHPPPQI

      651                                                        700
AAV_2 LIKNTPVPA NPSTTFSAAKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_7 LIKNTPVPA NPPEVFTPAKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_8 LIKNTPVPA DPPTTFNQSKL NSFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_1 LIKNTPVPA NPPAEFSATKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_3 MIKNTPVPA NPPTTFSPAKF ASFITQYSTG QVSVEIEWEL QKENSKRWNP
AAV_9 LIKNTPVPA DPPLTFNQAKL NSFITQYSTG QVSVEIEWEL QKENSKRWNP

      701                                                739
AAV_2 EIQYTSNYNK SVNVDFTVDT NGVYSEPRPI GTRYLTRNL
AAV_7 EIQYTSNFEK QTGVDFAVDS QGVYSEPRPI GTRYLTRNL
AAV_8 EIQYTSNYYK STSVDFAVNT EGVYSEPRPI GTRYLTRNL
AAV_1 EVQYTSNYAK SANVDFTVDN NGLYTEPRPI GTRYLTRPL
AAV_3 EIQYTSNYNK SVNVDFTVDT NGVYSEPRPI GTRYLTRNL
AAV_9 EIQYTSNYYK STNVDFAVNT EGVYSEPRPI GTRYLTRNL
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SEQUENCE LISTING

<110> The Trustees of the University of Pennsylvania
Rader, Daniel J.
Wilson, James M.

<120> Methods and Compositions for Lowering Total Cholesterol Levels and Treatment of Heart Disease

<130> UPN-P3084PCT

<150> US 60/465,293
<151> 2003-04-25

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20 25 30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160

Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190

Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly
195 200 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270

Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285

Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300

Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320

Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350

Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365

Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400

Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
405 410 415

Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430

Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr
435 440 445

Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser Arg Leu Gln Phe Ser Gln

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    450                 455                 460

Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly
465                 470                 475                 480

Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp Asn Asn
                485                 490                 495

Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly
            500                 505                 510

Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
        515                 520                 525

Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys
    530                 535                 540

Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
545                 550                 555                 560

Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln Tyr
                565                 570                 575

Gly Ser Val Ser Thr Asn Leu Gln Arg Gly Asn Arg Gln Ala Ala Thr
            580                 585                 590

Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp Gln Asp
        595                 600                 605

Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr
    610                 615                 620

Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys
625                 630                 635                 640

His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn
                645                 650                 655

Pro Ser Thr Thr Phe Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln
            660                 665                 670

Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys
        675                 680                 685

Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr
    690                 695                 700

Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr
705                 710                 715                 720

Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
                725                 730                 735
```

<210> 2
<211> 737
<212> PRT
<213> capsid protein of adeno-associated virus serotype 7

<400> 2

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Ala Lys Lys Arg
130 135 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145 150 155 160

Gly Lys Lys Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln
165 170 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro
180 185 190

Pro Ala Ala Pro Ser Ser Val Gly Ser Gly Thr Val Ala Ala Gly Gly
195 200 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn
210 215 220

Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225 230 235 240

```
Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

Leu Tyr Lys Gln Ile Ser Ser Glu Thr Ala Gly Ser Thr Asn Asp Asn
            260                 265                 270

Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Lys Leu Arg Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn
                325                 330                 335

Asn Leu Thr Ser Thr Ile Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn
    370                 375                 380

Gly Ser Gln Ser Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr Ser
                405                 410                 415

Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ala
        435                 440                 445

Arg Thr Gln Ser Asn Pro Gly Gly Thr Ala Gly Asn Arg Glu Leu Gln
    450                 455                 460

Phe Tyr Gln Gly Gly Pro Ser Thr Met Ala Glu Gln Ala Lys Asn Trp
465                 470                 475                 480

Leu Pro Gly Pro Cys Phe Arg Gln Gln Arg Val Ser Lys Thr Leu Asp
                485                 490                 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
            500                 505                 510

Leu Asn Gly Arg Asn Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
```

515 520 525

His Lys Asp Asp Glu Asp Arg Phe Phe Pro Ser Ser Gly Val Leu Ile
530 535 540

Phe Gly Lys Thr Gly Ala Thr Asn Lys Thr Thr Leu Glu Asn Val Leu
545 550 555 560

Met Thr Asn Glu Glu Glu Ile Arg Pro Thr Asn Pro Val Ala Thr Glu
565 570 575

Glu Tyr Gly Ile Val Ser Ser Asn Leu Gln Ala Ala Asn Thr Ala Ala
580 585 590

Gln Thr Gln Val Val Asn Asn Gln Gly Ala Leu Pro Gly Met Val Trp
595 600 605

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
610 615 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625 630 635 640

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
645 650 655

Ala Asn Pro Pro Glu Val Phe Thr Pro Ala Lys Phe Ala Ser Phe Ile
660 665 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
675 680 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
690 695 700

Asn Phe Glu Lys Gln Thr Gly Val Asp Phe Ala Val Asp Ser Gln Gly
705 710 715 720

Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 3
<211> 738
<212> PRT
<213> capsid protein of adeno-associated virus serotype 8

<400> 3

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145 150 155 160

Gly Lys Lys Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln
165 170 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro
180 185 190

Pro Ala Ala Pro Ser Gly Val Gly Pro Asn Thr Met Ala Ala Gly Gly
195 200 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
210 215 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225 230 235 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
245 250 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ala Thr Asn Asp
260 265 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
275 280 285

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Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Ser Phe Lys Leu Phe Asn
305                 310                 315                 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Thr Tyr
                405                 410                 415

Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala Asn Thr Gln Thr Leu Gly
    450                 455                 460

Phe Ser Gln Gly Gly Pro Asn Thr Met Ala Asn Gln Ala Lys Asn Trp
465                 470                 475                 480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Thr Gly
                485                 490                 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Ala Gly Thr Lys Tyr His
            500                 505                 510

Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro Gly Ile Ala Met Ala Thr
        515                 520                 525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Asn Gly Ile Leu Ile
    530                 535                 540

Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn Ala Asp Tyr Ser Asp Val
545                 550                 555                 560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
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565 570 575

Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu Gln Gln Gln Asn Thr Ala
580 585 590

Pro Gln Ile Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
595 600 605

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
610 615 620

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
625 630 635 640

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
645 650 655

Pro Ala Asp Pro Pro Thr Thr Phe Asn Gln Ser Lys Leu Asn Ser Phe
660 665 670

Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
675 680 685

Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
690 695 700

Ser Asn Tyr Tyr Lys Ser Thr Ser Val Asp Phe Ala Val Asn Thr Glu
705 710 715 720

Gly Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
725 730 735

Asn Leu

<210> 4
<211> 735
<212> PRT
<213> capsid protein of adeno-associated virus serotype 1

<400> 4

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

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Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145                 150                 155                 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
            180                 185                 190

Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
            260                 265                 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
        275                 280                 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
    290                 295                 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305                 310                 315                 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
                325                 330                 335
```

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
340 345 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
355 360 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
370 375 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385 390 395 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
405 410 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
420 425 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
435 440 445

Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
450 455 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
485 490 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
500 505 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
515 520 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
530 535 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
545 550 555 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
565 570 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
580 585 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His

610 615 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625 630 635 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
690 695 700

Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
705 710 715 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro
725 730 735

<210> 5
<211> 736
<212> PRT
<213> capsid protein of adeno-associated virus serotype 3

<400> 5

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Val Pro Gln Pro
20 25 30

Lys Ala Asn Gln Gln His Gln Asp Asn Arg Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Ile Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Gly
130 135 140

Ala Val Asp Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Val Gly
145 150 155 160

Lys Ser Gly Lys Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
180 185 190

Ala Ala Pro Thr Ser Leu Gly Ser Asn Thr Met Ala Ser Gly Gly Gly
195 200 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270

Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285

Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300

Gly Phe Arg Pro Lys Lys Leu Ser Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320

Arg Gly Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335

Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350

Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365

Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400

Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Thr Phe Glu
405 410 415

Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430

Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg Thr
435 440 445

Gln Gly Thr Thr Ser Gly Thr Thr Asn Gln Ser Arg Leu Leu Phe Ser
450 455 460

Gln Ala Gly Pro Gln Ser Met Ser Leu Gln Ala Arg Asn Trp Leu Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Leu Ser Lys Thr Ala Asn Asp Asn
485 490 495

Asn Asn Ser Asn Phe Pro Trp Thr Ala Ala Ser Lys Tyr His Leu Asn
500 505 510

Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys
515 520 525

Asp Asp Glu Glu Lys Phe Phe Pro Met His Gly Asn Leu Ile Phe Gly
530 535 540

Lys Glu Gly Thr Thr Ala Ser Asn Ala Glu Leu Asp Asn Val Met Ile
545 550 555 560

Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu Gln
565 570 575

Tyr Gly Thr Val Ala Asn Asn Leu Gln Ser Ser Asn Thr Ala Pro Thr
580 585 590

Thr Gly Thr Val Asn His Gln Gly Ala Leu Pro Gly Met Val Trp Gln
595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
610 615 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625 630 635 640

Lys His Pro Pro Pro Gln Ile Met Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asn Pro Pro Thr Thr Phe Ser Pro Ala Lys Phe Ala Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln

675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
690 695 700

Tyr Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val
705 710 715 720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<210> 6
<211> 736
<212> PRT
<213> capsid protein of adeno-associated virus serotype 9

<400> 6

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
145 150 155 160

Lys Ser Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
180 185 190

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Glu Ala Pro Ser Gly Leu Gly Pro Asn Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp Asn
            260                 265                 270

Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Thr Asn Glu Gly Thr Lys Thr Ile Ala Asn
                325                 330                 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn
    370                 375                 380

Gly Ser Gln Ala Leu Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Thr
                405                 410                 415

Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Val
        435                 440                 445

Arg Thr Gln Thr Thr Gly Thr Gly Gly Thr Gln Thr Leu Ala Phe Ser
    450                 455                 460
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Gln Ala Gly Pro Ser Ser Met Ala Asn Gln Ala Arg Asn Trp Val Pro
465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Thr Asn Gln Asn
485 490 495

Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Ala Lys Phe Lys Leu Asn
500 505 510

Gly Arg Asp Ser Leu Met Asn Pro Gly Val Ala Met Ala Ser His Lys
515 520 525

Asp Asp Glu Asp Arg Phe Phe Pro Ser Ser Gly Val Leu Ile Phe Gly
530 535 540

Lys Gln Gly Ala Gly Asn Asp Gly Val Asp Tyr Ser Gln Val Leu Ile
545 550 555 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Glu
565 570 575

Tyr Gly Ala Val Ala Ile Asn Asn Gln Ala Ala Asn Thr Gln Ala Gln
580 585 590

Thr Gly Leu Val His Asn Gln Gly Val Ile Pro Gly Met Val Trp Gln
595 600 605

Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
610 615 620

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
625 630 635 640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
645 650 655

Asp Pro Pro Leu Thr Phe Asn Gln Ala Lys Leu Asn Ser Phe Ile Thr
660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
690 695 700

Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu Gly Val
705 710 715 720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<210> 7

<211> 4721
<212> DNA
<213> adeno-associated virus serotype 7

<400> 7
ttggccactc cctctatgcg cgctcgctcg ctcggtgggg cctgcggacc aaaggtccgc 60
agacggcaga gctctgctct gccggcccca ccgagcgagc gagcgcgcat agagggagtg 120
gccaactcca tcactagggg taccgcgaag cgcctcccac gctgccgcgt cagcgctgac 180
gtaaatcacg tcatagggga gtggtcctgt attagctgtc acgtgagtgc ttttgcgaca 240
ttttgcgaca ccacgtggcc atttgaggta tatatggccg agtgagcgag caggatctcc 300
attttgaccg cgaaatttga acgagcagca gccatgccgg gtttctacga gatcgtgatc 360
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Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
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Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
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Arg Glu Lys Leu Val Gln Thr Ile Tyr Arg Gly Val Glu Pro Thr Leu
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Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
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Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
145                 150                 155                 160

Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu Tyr Ile
                165                 170                 175

Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala Gln His
            180                 185                 190

Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Leu Asn
        195                 200                 205

Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
    210                 215                 220

Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser Glu Lys
225                 230                 235                 240

Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
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Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
            260                 265                 270

Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly Pro Ser
        275                 280                 285

Leu Pro Ala Asp Ile Lys Thr Asn Arg Ile Tyr Arg Ile Leu Glu Leu
    290                 295                 300

Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly Trp Ala
305                 310                 315                 320

Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
                325                 330                 335

Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala Val Pro
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Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
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Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
385                 390                 395                 400

Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
                405                 410                 415

Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
            420                 425                 430

Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
        435                 440                 445

Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr Lys Gln
    450                 455                 460

Glu Val Lys Glu Phe Phe Arg Trp Ala Ser Asp His Val Thr Glu Val
465                 470                 475                 480

Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Ser Lys Arg Pro Ala
                485                 490                 495

Pro Asp Asp Ala Asp Ile Ser Glu Pro Lys Arg Ala Cys Pro Ser Val
            500                 505                 510

Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp Phe Ala
        515                 520                 525

Asp Arg Tyr Gln Asn Lys Cys Ser Arg His Ala Gly Met Ile Gln Met
    530                 535                 540

Leu Phe Pro Cys Lys Thr Cys Glu Arg Met Asn Gln Asn Phe Asn Ile
545                 550                 555                 560

Cys Phe Thr His Gly Val Arg Asp Cys Leu Glu Cys Phe Pro Gly Val
                565                 570                 575

Ser Glu Ser Gln Pro Val Val Arg Lys Lys Thr Tyr Arg Lys Leu Cys
            580                 585                 590

Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys Ser Ala
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Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu Gln
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<211> 625
<212> PRT
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20 25 30

Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Arg Asn Leu Ile
35 40 45

Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
50 55 60

Val Gln Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
65 70 75 80

Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Leu His Val Leu Val Glu
85 90 95

Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
100 105 110

Arg Glu Lys Leu Gly Pro Asp His Leu Pro Ala Gly Ser Ser Pro Thr
115 120 125

Leu Pro Asn Trp Phe Ala Val Thr Lys Asp Ala Val Met Ala Pro Ala
130 135 140

Gly Gly Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu
145 150 155 160

Pro Lys Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Glu
165 170 175

Tyr Ile Ser Ala Cys Leu Asn Leu Ala Glu Arg Lys Arg Leu Val Ala
180 185 190

Gln His Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn
195 200 205

Leu Asn Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala
210 215 220

Arg Tyr Met Glu Leu Val Gly Trp Leu Val Asp Arg Gly Ile Thr Ser
225 230 235 240

Glu Lys Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn
245 250 255

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Ala Ala Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala
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Gly Lys Ile Met Ala Leu Thr Lys Ser Ala Pro Asp Tyr Leu Val Gly
        275                 280                 285

Pro Ser Leu Pro Ala Asp Ile Thr Gln Asn Arg Ile Tyr Arg Ile Leu
    290                 295                 300

Ala Leu Asn Gly Tyr Asp Pro Ala Tyr Ala Gly Ser Val Phe Leu Gly
305                 310                 315                 320

Trp Ala Gln Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly
                325                 330                 335

Pro Ala Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Ala
            340                 345                 350

Val Pro Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe
        355                 360                 365

Asn Asp Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met
    370                 375                 380

Thr Ala Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys
385                 390                 395                 400

Val Arg Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr
                405                 410                 415

Pro Val Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly
            420                 425                 430

Asn Ser Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe
        435                 440                 445

Lys Phe Glu Leu Thr Arg Arg Leu Glu His Asp Phe Gly Lys Val Thr
    450                 455                 460

Lys Gln Glu Val Lys Glu Phe Phe Arg Trp Ala Ser Asp His Val Thr
465                 470                 475                 480

Glu Val Ala His Glu Phe Tyr Val Arg Lys Gly Gly Ala Ser Lys Arg
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Pro Ala Pro Asp Asp Ala Asp Lys Ser Glu Pro Lys Arg Ala Cys Pro
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Ser Val Ala Asp Pro Ser Thr Ser Asp Ala Glu Gly Ala Pro Val Asp
        515                 520                 525
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Asn Ile Cys Phe Thr His Gly Val Arg Asp Cys Ser Glu Cys Phe Pro
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Gly Val Ser Glu Ser Gln Pro Val Val Arg Lys Arg Thr Tyr Arg Lys
            580                 585                 590

Leu Cys Ala Ile His His Leu Leu Gly Arg Ala Pro Glu Ile Ala Cys
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Ser Ala Cys Asp Leu Val Asn Val Asp Leu Asp Asp Cys Val Ser Glu
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